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CONSPICUOUS HISTOMORPHOLOGICAL ANOMALIES IN THE HIPPOCAMPAL FORMATION OF RATS EXPOSED PRENATALLY TO A COMPLEX SEQUENCED MAGNETIC FIELD WITHIN THE NANOTESLA RANGE[1]

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Summary.—The brains of adult rats, exposed prenatally to one of four intensities (between 10 nanoTesla and 1.2 microTesla) of either a frequency-modulated magnetic field or a complex sequenced field designed to affect brain development, were examined histologically. Although from each intensity some rats that had been exposed to the complex sequenced magnetic field showed minor anomalies, those exposed to intensities between 30 nT and 180 nT exhibited conspicuous anomalous organizations of cells within the hippocampal formation. In other studies, rats that had been exposed during their entire prenatal development to the complex sequenced field displayed significantly more activity in the open field and poorer spatial memory during maze learning. Photomicrographs are shown of one conspicuous morphological anomaly within the right hippocampus of an adult rat exposed prenatally to the complex sequenced magnetic field with intensities between .3 mG and .5 mG (30 nT to 50 nT). The results suggest that complex magnetic fields, whose temporal structures approach the time constants of normal biochemical processes, can permanently alter the development of the brain.

From a quantal perspective all chemical reactions from which all cellular activities are created also display electromagnetic equivalents. Chemical reactions and interactions are determined by the complexity of their spatial configurations or molecular structures. We have hypothesized that electromagnetic interactions with cells should be determined by the complexity of their temporal structure.

The developing organism begins as a single cell and through repeated bifurcations or binomial divisions results in a living organism. If we assume a single cell is 5 micrometers in radius, has the specific gravity of water, and duplicates without significant cell mortality, then about 40 divisions would be required to obtain a 1-kg mass. For the rat, about 35 of these divisions occur within only 3 wk. during prenatal development.

The expression of genes during this period can be influenced by innumerable teratogenic agents that can influence both the migrational and postmigrational organization of neurons. In general, expression is affected by the translation of genetic potential from DNA to RNA sequences. The precise stacking of base-pairs of RNA ultimately affects protein synthesis. The time

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required for this stacking is about 1 msec. and involves energies in the order of 10^{-20} joules which is generated by normal action potentials (Wei, 1969).

We reasoned that complex magnetic fields, if the temporal parameters were rationally designed, could influence morphological expression during prenatal development. Our recent, unpublished pilot studies (Lee, St-Pierre, & Persinger) with micro-array analyses of gene expression in the brains of adult C57 mice exposed to this complex sequenced field for about 3 wk. supported the validity of our assumptions. The brains of these mice showed up-regulation of genes: prealbumin (NCBI No. H3005F03), p96 (H30884D02) which is phosphorylated in response to mitogenic growth factor stimulation, and C-P-45-(16) alpha (H3026E09). Genes that were down-regulated were PIP5Kgamma (H3121H08), a Type 1 phosphotidylinositol 4-phosphate 5 kinase gene, and GDAP1 (H3058B12), a ganglioside-induced differentiation-associated protein.

In previous studies we found rats that had been exposed prenatally to a complex sequenced magnetic field composed of essential neurophysiological patterns displayed significantly different behaviors in the open field compared to rats that had been exposed to more simple frequency-modulated field or to fields designed to imitate sudden storm commencements (sudden impulses) within the earth's magnetic field (Persinger, St-Pierre, & Koren, 2001). The rats that had been exposed to this complex sequenced field during their prenatal development required more trials to learn a spatial maze (McKay, St-Pierre, & Persinger, 2003). Its successful acquisition is strongly influenced by the integrity of the hippocampus, particularly the "micro-organization" within the interface between the dentate gyrus and the CA3/CA4 region.

The present study reports the first examination of the brains of rats exposed to the frequency-modulated or the complex sequenced magnetic fields. We predicted that the behavioral deficits of the rats required the existence of discrete morphological or chemical changes within the hippocampal formation or its tract systems. Considering the magnitude of the behavioral changes in these rats, these morphological anomalies were predicted to be discernable by light microscopy.

Method

Subjects

A total of 16 brains (2 per condition) were extracted from rats that had been exposed during their entire prenatal development to one of four intensities (600 nT to 1.2 microT, 90 nT to 200 nT, 30 nT to 50 nT, and 5 nT to 20 nT) of either a frequency-modulated field or a complex sequenced magnetic field. The rationale and details of the fields and a description of the exposure equipment have been presented elsewhere (Persinger, *et al.*, 2001).

In brief, the frequency-modulated field was presented continuously while the complex sequenced field was presented for 10 sec. once every 40 sec. The duration of each point that comprised the frequency-modulated pattern was 3 msec. The complex sequenced magnetic field was composed of 50 blocks wherein each block was presented for 200 msec. Every second block was a pattern known to enhance long-term potentiation in hippocampal slices (McKay, Persinger, & Koren, 2000). The duration for each of the 10,000 points that composed this entire field was 1 msec. Rats were removed within two days of birth and, after weaning, maintained three per (same sex) cage. Food and water were available *ad libitum*. The rooms were temperature controlled (19–21°C), and the light:dark cycle was 12:12, with light onset at 08 hr. local time.

At approximately 200 days of age the rats were decapitated and the brains were removed, fixed in ethanol-formalin-acetic acid, and processed. Ten micrometer coronal sections between the posterior commissure and anterior commissure were stained with toluidine blue O. Additional staining included haematoxylin and eosin and the periodic-Schiff-myelin stain from Humason (1967). Sections were examined at 40×, 100×, 400×, and 1000× (oil immersion). Only qualitative conspicuous anomalies were recorded. The characteristics of the anomaly were examined by the second author with the experience of approximately 500 adult rat brains during the last 35 years.

Results

The results were qualitatively conspicuous. At the coronal level where the ventromedial hypothalamus is a clear marker, aberrant rows of granule cells were found within the interface between CA3/CA4 and the dentate gyrus of more than half of the rats exposed to the complex sequenced field, while this pattern was not apparent in the hippocampal formations of the rats exposed to the frequency-modulated field. The more intense examples were evident in brains of rats that had been exposed to the 90 nT to 200 nT (.9 mG to 2 mG) and 30 nT to 50 nT (.3 mG to .5 mG) fields. The rats that had been exposed to the highest intensity (5 mG to 12 mG) or the lowest intensity (.05 mG to .2 mG) fields did not exhibit the incidence or prevalence of these conspicuous anomalies.

Fig. 1 shows the most extreme example of the anomalies found within a large region of the hippocampus of a rat that had been exposed to the 30 nT to 50 nT complex sequenced field. Close inspection at 1000× also indicated the glial cells and normal appearing neurons were clustered around irregularly shaped capillaries. The extent of the densely stained regions, which at higher power magnification were due to the increased density of glial cells and oddly arranged neurons, covered about 30% of the cross-sectional area of this component of the hippocampus. However, the hippocampal forma-

tion within the left hemisphere appeared normal within the limits of light microscopy.

The rats that had been exposed to the complex sequenced fields, particularly the low intensities, also exhibited more vacuoles and holes within the white matter of the fimbria compared to rats exposed to the frequency-modulated fields. The fimbria fibers were also more irregular and the typical rows of oligodendroglia nuclei were less organized. Other qualitative anomalies occurred within the cerebellums. Rats that had been exposed to either the frequency-modulated or complex sequence fields with intensities between 30 nT and 50 nT showed small regions (about 50 μm in width) of double rows of Purkinje cells.

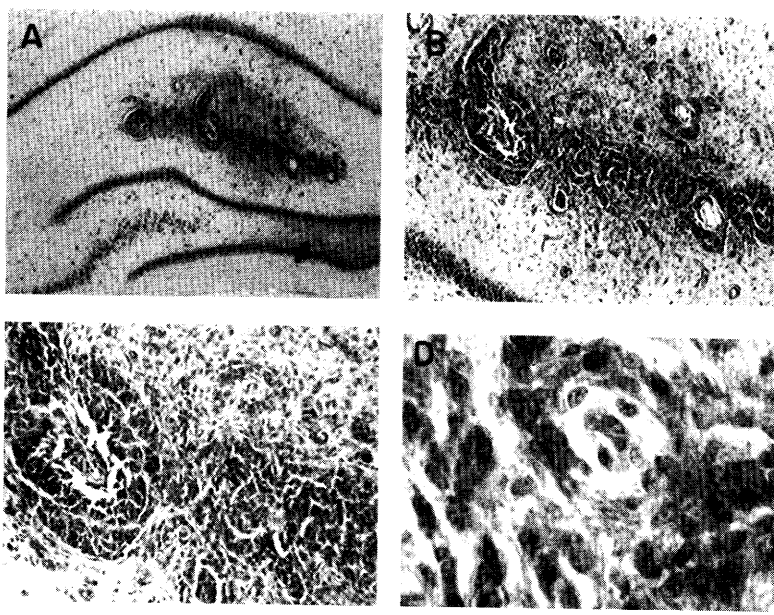

FIG. 1. Photomicrographs of an adult rat exposed during prenatal development to 30 nT to 50 nT complex sequenced magnetic field: (A) 40×) increased density of granule cells in the right dentate gyrus (arrow) and conspicuous heterotopia in the hippocampal formation. (B) 100× of heterotopic area in the hippocampus. (C) 200× of rosettes of neurons and glial cells. (D) 400× of glial cell organization within the rosettes.

DISCUSSION

During the last 35 years of research with various teratogenic stimuli (from ionizing radiation to various chemical treatments) involving several hundreds of rat brains, the second author has not observed the type of morphological anomaly expressed by adult rats that had been exposed prenatally

to the complex sequenced magnetic field. Most teratogenic treatments, such as prenatal or postnatal exposures to between 1 and 2 Joules/kg (Gray) of ionizing radiation, affect the entire volume of the brain and induce ectopic tissue or disorganized cytological arrangements throughout the brain. Usually exposures to teratogens affect the portion of the brain that was undergoing peaks in migration or mitosis at the time of treatment.

That the present effect was associated with a particular complexity of the magnetic field rather than the presence of any complex field was indicated by the absence of these morphological anomalies in brains of rats that had been exposed to the frequency-modulated field with comparable intensities. The specificity of this effect within the hippocampal formation indicates a more precise mechanism through which the influence of the complex sequence magnetic field was mediated. Such cellular anomalies, ranging from subtle to conspicuous within the hippocampal formation, would be consistent with the significant deficits in spatial memory and learning displayed by the rats that had been exposed prenatally to the complex sequenced magnetic fields but not other complex fields (McKay, *et al.*, 2003).

The rats exposed to the 30 nT to 50 nT fields also displayed brain weights that were about 2% heavier (St-Pierre, 2001) than the brains from other conditions. If we assume that a rat brain is about 2 cc in volume and each neuron is one-billionth of a cubic centimetre, then the average rat brain would contain about 2 billion cells. A 2% increase in weight, assuming the specific gravity of water, would be equivalent to about 40 million neurons. On the other hand, given the usual assumption that about 10% of brain volume is extracellular fluid, then a 2% increase in brain weight exclusively within the extracellular fluid may have contributed to the increased numbers of "vacuoles."

We suggest this field may have influenced the small subpopulation of neurons, probably at the site of progenitor cells within ventricular lining that have the capacity to display mitosis within the adult rat brain. According to Derrick, York, and Martinez (2000) increased neurogenesis of granule cells occurs in the adult dentate gyrus of rats following stimulation of mossy fibers with currents sufficient to produce long-term potentiation (LTP). It would be intriguing were the same pulse pattern known to affect long-term potentiation in adult rat hippocampuses (McKay, *et al.*, 2000) also affected the migration to and differentiation of neuroblasts within this structure. Their commonality would suggest at least partial validity for Burr's concept (1972) of fundamental "fields" or "blueprints" that are employed as reiterative algorithms connecting the stages of ontogeny. Interestingly, mossy fibers within this same region of epileptic brains of both humans and rats are well known to display reactive synaptogenesis (Houser, 1992; Parent, Yu, Leibowitz, Geschwind, Sloviter, & Lowenstein, 1997).

Whether or not the prenatal exposures to these fields affected the "timing" of genes such that neuronal mitosis and the concomitant gliosis (Zang, Kelly, Zerillo, Jaworski, & Hockfield, 1998) and angiogenesis actually occurred in adulthood is not clear and would require examination of younger brains. The spatial organization of the anomaly in Fig. 1 would also be consistent with subtle differences in the expression of the lecticans or how they interact with carbohydrate and protein ligands in the extracellular matrix (Yamaguchi, 2000; Ogawa, Hagihara, Suzuki, & Yamaguchi, 2001). The features of the anomaly are more typical of heterotopias than of gene-triggered formation of tumors. Although there were indicators of unusual angiogenesis, the neuronal soma within the anomalous areas appeared normal, and there was no evidence of macrophagic activity or edema.

Lecticans (Yamaguchi, 2000) are a family of chondroitin sulphates characterized by different combinations of hyaluronates and lecticans bound to core proteins. They are an important component of ground substance. Different proteoglycans act as regulators of cell migration, axonal path finding, synaptogenesis, and structural plasticity.

Lecticans because of their size and massive surround of charged particles might be considered "electric sinks" for these effects. If we assume a changing field of 50 nT per .001 sec. and a curl E (electric field) over a .01-m surface, then the potential would be 10 microV/m or .1 microV/cm. Extracellular conductive fluids with resistances of 100 ohm/cm would allow transient electric currents in the order of a nanoA. Were the small spaces containing lecticans very conductive, then currents with intensities of only 1 nA/cm-squared focused to smaller cross-sectional areas of 1 nA/micrometer-squared would increase the movement of electrons from these polyanionic nets that extends the functional neuronal membrane from 10 nm to 100 or more nm by a factor of about 100 million.

The milder anomalies as well as the one presented in Fig. 1 occurred in rats that were exposed to field strengths between 0.5 and 1.5 mG of the complex sequenced fields. If this nonlinear relationship between intensity and effect is generalizable, then traditional assumptions of dose dependence to determine the safety of similar fields for the developing fetus might be re-examined. These configurations should become more predominant as the complexity of electromagnetic emissions from various technologies escalates. The superimpositions of millions of MHz and GHz signals will produce higher order matrices of beats and interference patterns, with functional point durations within the 1-msec. range, to which the developing organism will be constantly exposed.

This "window" effect might also suggest that a quanta associated with this intensity of field, once translated into its effective energy at the molecular or cellular level, is required to initiate the change. Like the size and

shape of molecules proximal to receptors such that structures that are too large or too small will not be sequestered, only "quanta" of specific temporal geometries would be effective. Although stronger intensities with simple shapes could be effective, other mechanisms might be involved. The differences between the weak complex and strong simple time-varying magnetic fields on developing systems might be comparable to the differences between high affinity and low affinity receptors.

The timing and composition of the complex sequence field were designed by the second author to affect gene expression (Persinger, *et al.*, 2001). However, we did not measure gene expression directly in the present study. Since the completion of our study Michele, Barresi, Kanagawa, Saito, Cohn, Satz, Dollar, Nishino, Kelley, Somer, Straub, Matthews, Moore, and Campbell (2002) have reported posttranslational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. These rodents also showed abnormal neuronal migration in the hippocampus and cerebellum. The mechanism appeared to involve disruptions within the extracellular matrix proteins. At least three distinct mammalian gene functions with a convergent posttranslational pathway were implicated.

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